# Biosensors Made from Carbon and Polymer Composite

# Micro-electromechanical Systems (MEMS)

**Interim Report**



**ONR Award No. N00014-03-1-0893**

**for the work performed June 21 – Dec. 21, 2003**

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# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT DATE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 21-01-2004 | Interim | June 2003 – January 2004 |

**4. TITLE AND SUBTITLE**

Biosensors Made from Carbon and Polymer Composite Micro-electromechanical Systems (MEMS)

**5a. CONTRACT NUMBER**
N/A

**5b. GRANT NUMBER**
N00014-03-1-0893

**5c. PROGRAM ELEMENT NUMBER**
N/A

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**
N/A

**5e. TASK NUMBER**
I, II, IV

**5f. WORK UNIT NUMBER**
N/A

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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Springfield, MO 65804

**8. PERFORMING ORGANIZATION REPORT NUMBER**
#1

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

Office of Naval Research Regional Office Chicago
230 South Dearborn, Room 380
Chicago, IL 60604-1595

**10. SPONSOR/MONITOR'S ACRONYM(S)**
ONR

**11. SPONSORING/MONITORING AGENCY REPORT NUMBER**
N/A

**12. DISTRIBUTION AVAILABILITY STATEMENT**

Approved for Public Release; Distribution is Unlimited

**13. SUPPLEMENTARY NOTES**

Research in collaboration with Brewer Science, Inc. (Rolla, MO)

**14. ABSTRACT**

The goal of this project is to develop a new class of biosensors using carbon and polymer based micro-electromechanical systems (MEMS). This interim report discusses the progress to date on the individual tasks outlined in the proposal. Progress has included a study to engineer the appropriate carbon/polymer/metal cluster compositions which have vibration sensitive electronic properties. Initial imaging of structures created using microlithography and imaging of the carbon composite, biocompatible surface were done with SEM and AFM respectively. The methodology used for building antibody microarrays to detect specific molecular binding events was demonstrated. Preliminary work on a prototype for an antigen collection system has been accomplished.

**15. SUBJECT TERMS**

CPMEMS, Surface Acoustic Waves, Microstructure, Scanning Electron Microscope, Ion Implantation, Atomic Force Microscope, Electrical Conductivity, Microarray, Detection of Antibodies, Antigen Collection

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | Dr. Ryan Giedd |
| N/A | N/A | N/A | UU | 14 | 19b. TELEPONE NUMBER *(Include area code)* (417) 836-5131 |

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## I. Introduction

This project is divided up into four primary technical tasks. These tasks involve different aspects of the technology that will be utilized in the design and testing of carbon and polymer based micro-electromechanical systems (CPMEMS) and each task has its underlying objectives. It is the intent of this interim report to discuss the progress to date on the tasks outlined in the original proposal. The tasks are:

- Task I – Device Fabrication
- Task II – Antigen Platform Feasibility
- Task III – Developing Partnerships
- Task IV – Final Design and Testing

Task I and Task II have primary effort in the last six months and will be discussed in detail the following sections of this interim report. Task III and IV, which have primary effort in the months ahead, will also be discussed to some extent in the following sections.

## II. Description of Technical Research Progress

### a. Au/PSA composite material

The main objective of Task I – Device Fabrication is to engineer the appropriate carbon/polymer/metal cluster compositions which have electronic properties that are critically dependent on vibrations [1] or surface acoustic waves (SAW) [2]. A study was performed using the polymer poly(styrene-co-acrylonitrile) (PSA) and the metal gold (Au).

A set of varying-thickness Au-films were thermally evaporated onto poly(styrene-co-acrylonitrile) thin film surfaces. The Au/PSA bi-layer targets were then implanted with 50 keV N+ ions to a dose of $1x10^{16}$ ions/cm$^2$. The electrical conductivity, film microstructure, and elemental diffusion/mixing within the Au/PSA interface were evaluated. Electrical conductivity measurements of the implanted Au/PSA thin films show a sharp percolation behavior versus the pre-implant Au-film thickness with a percolation threshold near the nominal thickness of 44 Å.

Figure 1 below, is a comparison of sheet resistance (Ohms/sq) as a function of the pre-implant Au-thickness before and after ion implantation. Notice the sharp transition between 40 Å and 50 Å where the sheet resistance decreases over four orders of magnitude. In the sharp percolation region the materials have the appropriate microstructure that is ideal for sensitivity to vibration and will be useful for SAW devices.

Figure 2 shows the relative resistance change as a function of temperature for the implanted Au/PSA bi-layer thin films. Notice that the thicknesses below the transition exhibit semiconductor-like curves and the thicknesses above the transition have metallic curves again reinforcing that the materials is moving through a critical transition as the pre-implant thickness of gold is increased.

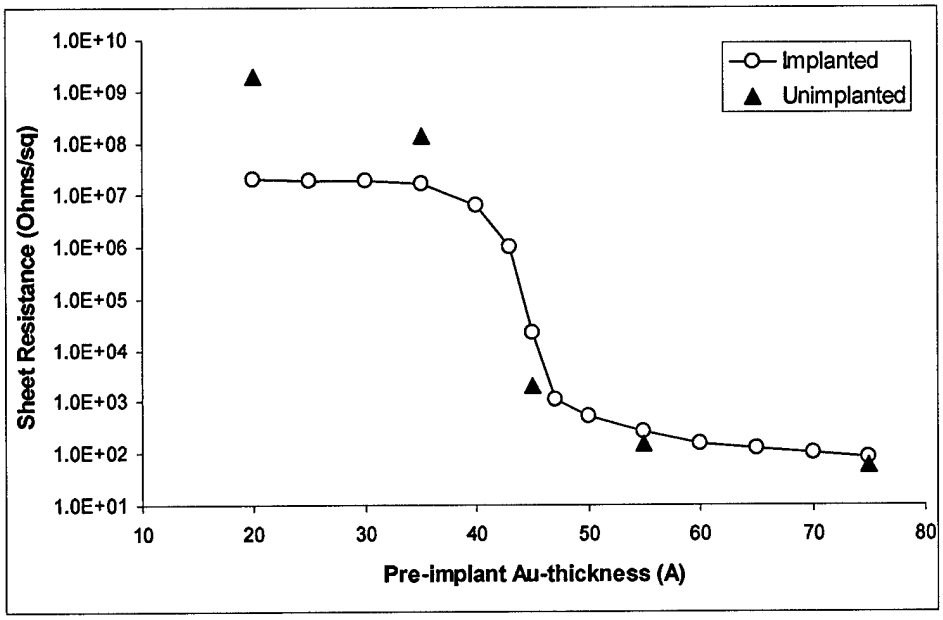


**Figure 1:** Sheet resistance vs. pre-implant Au-thickness

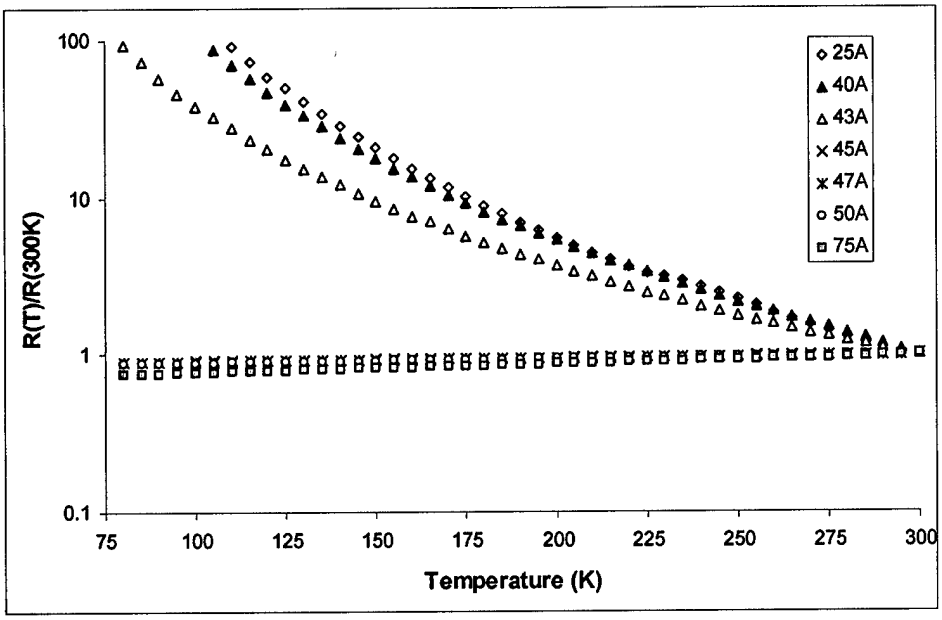


**Figure 2:** Relative resistance change as a function of temperature

Figure 3 shows, pictorially utilizing a scanning electron microscope (SEM), the microstructure evolution as the gold pre-implant thickness increases. When no Au is present the implanted polymer is a consistent hydrogen-depleted carbon network. It is this carbon network that is believed to make the polymer harder, more conductive, and more resistive to mechanical wear and chemicals.

When the Au film is very thin (20 Å) the implanted Au-particles are isolated from each other (~100 nm apart) and the resistivity is high (semiconductive-like material). Au particles are light colored and the implanted PSA regions are dark.

As the pre-implant Au-film grows thicker (45 Å), the initially separated Au-particles start to form Au-islands (clusters) and the distance between these conducting clusters gets smaller and smaller. This is the transition region or steep portion of Figure 1. The electrical conduction behavior is moving through a semiconductive to metallic transition.

As the Au-thickness continues to increase, these conducting islands start to overlap and finally form a nearly continuous Au-film (75 Å). As a result, the electrons are free to move within the continuous Au-film (metallic conduction).

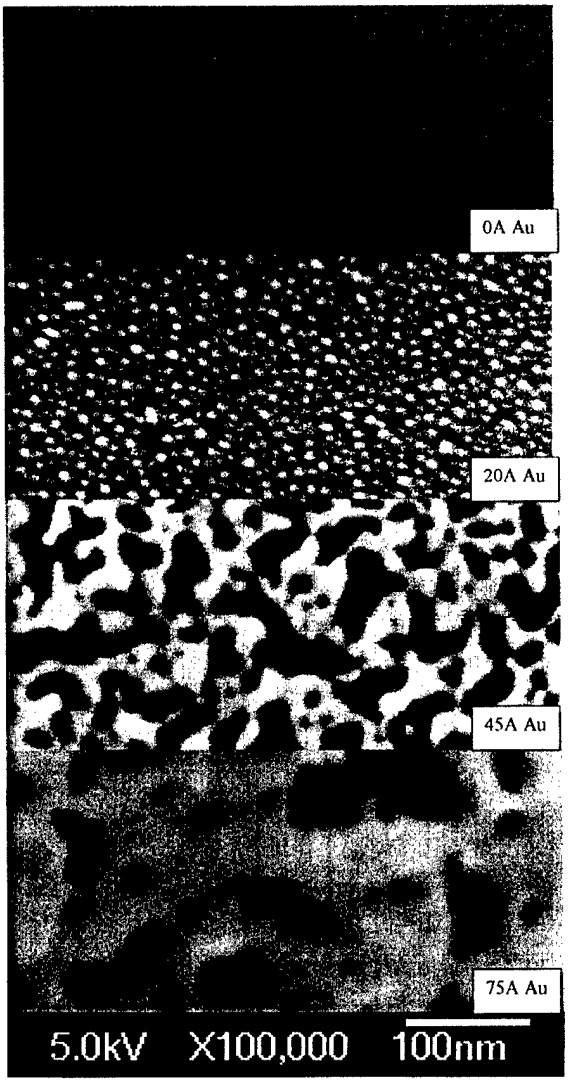


**Figure 3:** SEM images of the microstructure evolution

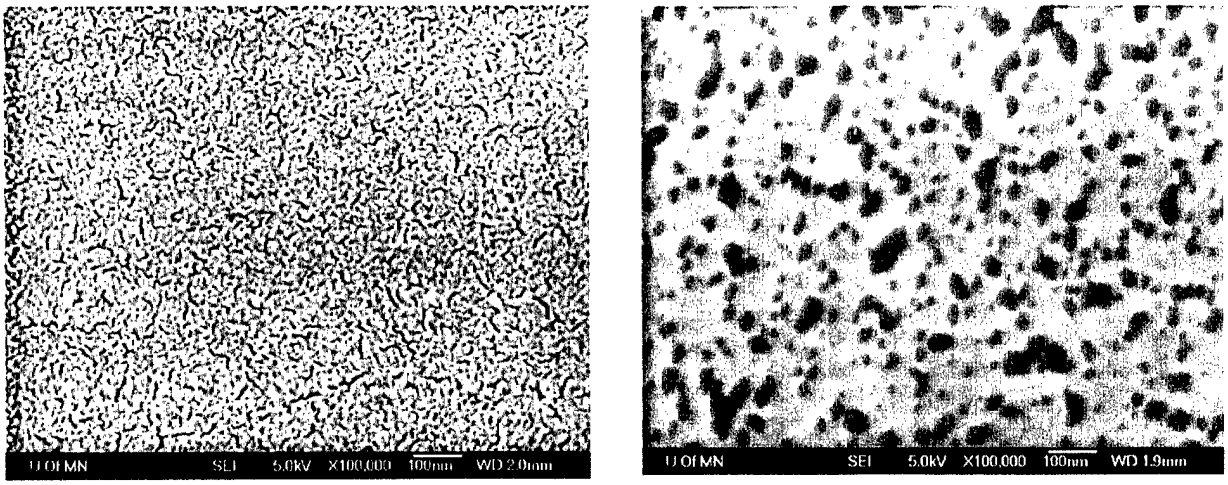


**Figure 4:** SEM images of the 75 Å sample before (left) and after (right) implantation

Figure 4 depicts the SEM images of Au-75Å/PSA thin film before (left) and after (right) ion implantation. The post treatment of the metal/polymer bi-layer system by the ion implantation process promotes the metal to polymer adhesion, by embedding the Au particles into a simultaneously created hydrogen-depleted carbon network. These images show why ion implantation is a critical process in producing these materials.

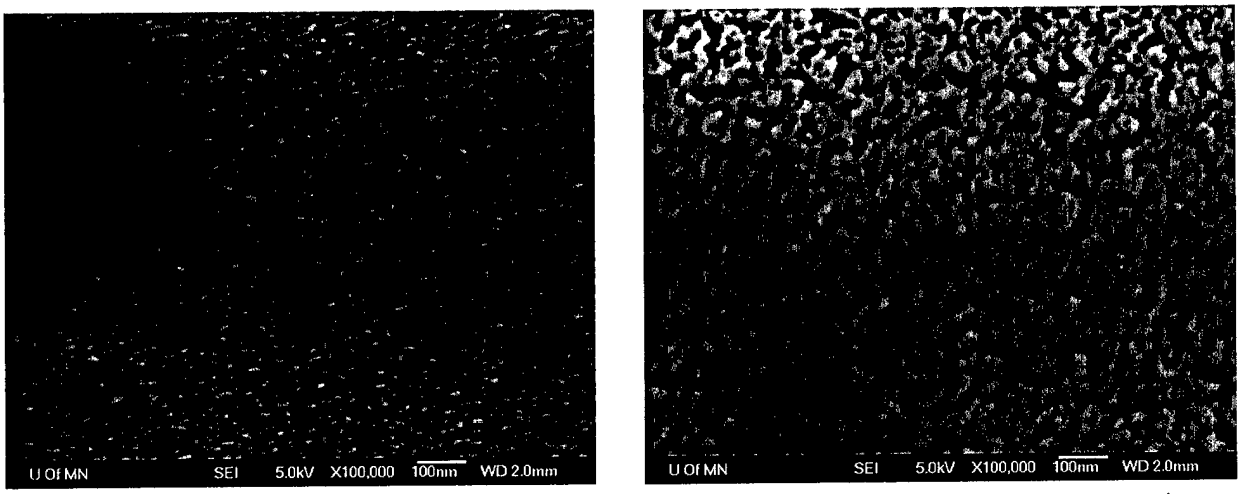


**Figure 5:** SEM images of samples in the transition region

Figure 5 shows SEM images of Au-40Å/PSA (left) and Au-45Å/PSA (right) after ion implantation. In the above images an intricate network of Au (light) and implanted PSA regions (dark) is evident. The images show that the gold cluster size is larger in the 45 Å sample versus the 40 Å sample. This is a very distinct change for such a small difference in pre-implant Au thickness, thus showing that we have precise control over the materials electrical properties.

Because of the microstructure and hopping conduction mechanism, that is highly sensitive to the relative proximity of the hop sites [3], these materials are sensitive to vibration and look very promising for sensors using surface acoustic waves.

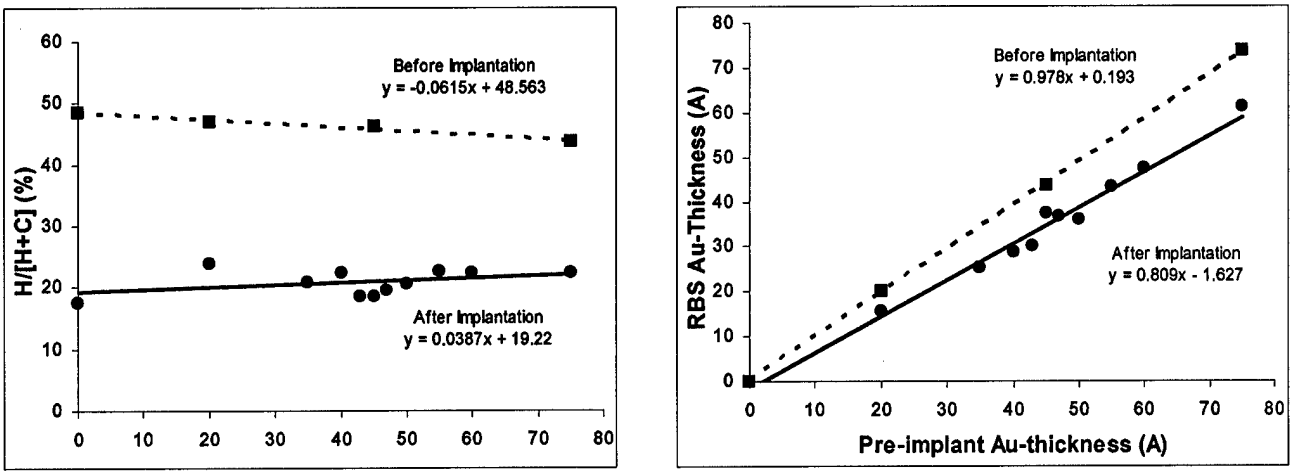


**Figure 6:** RBS and ERD data

The ion beam analysis techniques Rutherford Backscattering Spectrometry (RBS) and Elastic Recoil Detection (ERD) and were used to measure Au and H-content, respectively, in the targets. Figure 6 (left), shows the measured H-content in the Au/PSA films as a function of the pre-implant Au-thickness. The results show that ion implantation liberated H-content in the PSA ($C_{11}H_{11}N$) films by approximately 30% thereby creating the C-rich network.

Figure 6 (right), shows the measured Au-content in the Au/PSA films as a function of the pre-implant Au-thickness. The plot depicts an approximate 20% loss of Au during the implant process. This indicates that not the entire pre-implant Au layer is embedded into the PSA; some is sputtered off during ion implantation.

## b. Microlithography

Another major objective of Task I – Device Fabrication is to optimize the microlithography processes used to manufacture the carbon and polymer composite microelectromechanical systems (CPMEMS). Utilizing the JEOL 6360LV scanning electron microscope that was purchased with funds from our equipment budget, we are now able to fine-tune our microlithography processes and recipes to get the crisp fine features that are needed for building the CPMEMS.

Figure 7 depicts SEM images of gold patterns constructed through microlithography. They are of the wet etched metal layer used as the support structure and also used to make electrical connection to the sensor material (bridge structure). The image on the left shows an array of gold pads with varying separation distance. The pads are 125 μm x 125 μm and the thin vias (lines) are 10 μm in width. The image on the right is a higher magnification of just three of the gold pads from the left image. These SEM images confirm that our microlithography processes can produce the fine feature sizes needed for the CPMEMS.

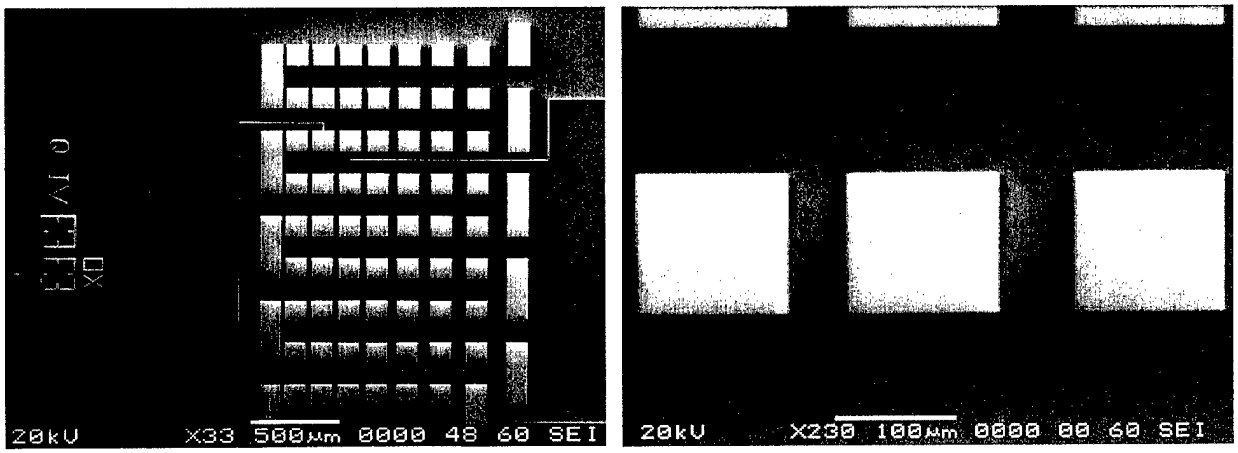


**Figure 7:** SEM images of microlithography

### c. AFM imaging of biocompatible surface

Utilizing the atomic force microscope (AFM) that was purchased from Veeco Metrology Group with funds from our equipment budget, we are now able to get a nano-scale interpretation of the surface of our carbon composite surfaces. Below in Figure 8 are some images taken of the Au/PSA composite material discussed in section IIa. The images on left show a 2D representation of the different height and frictional areas on the implanted Au-20Å/PSA sample surface. While the image on the right shows a 3D representation of the surface topography of the same sample. This instrument helps us to better understand the microstructural evolution of the Au/PSA composite materials as the pre-implant gold thickness is increased through the transition region. With the AFM we are able learn different types of information in addition to the SEM images. We can acquire 3D surface topography, frictional differences in areas on the surface, and indications of differences in height, to name a few.

Future work with this instrument will allow us to observe the carbon composite material as it is modified to bind with proteins and antibodies. It will also help determine the nature of antigens attached to the surface of the devices.

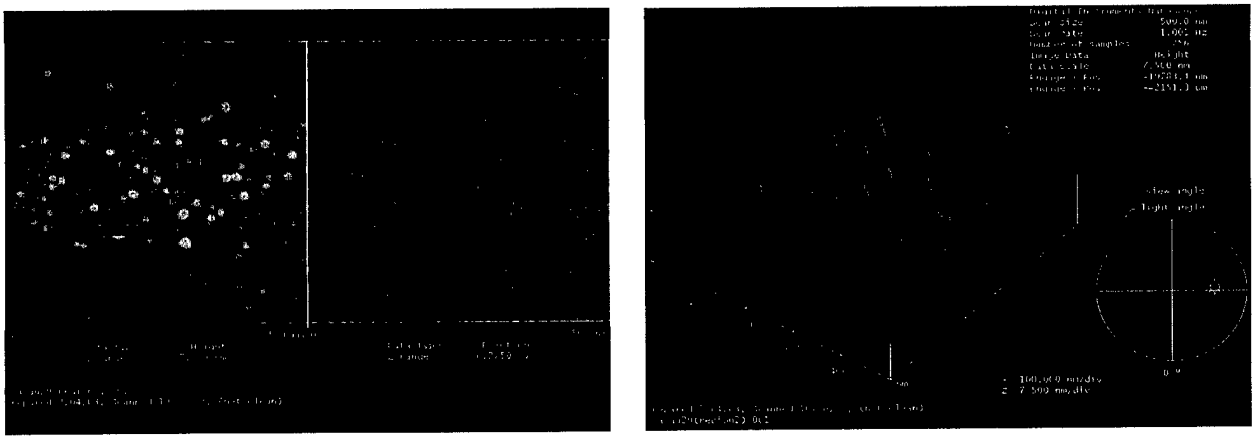

**Figure 8:** AFM images of Au/PSA composite material

## d. Development of procedure for self-assembled protein layer

The primary objective of Task II – Antigen Platform Feasibility is to develop the appropriate process to bond the proteins, antibodies, or oligonucleotides to the carbon surface of the CPMEMS.

Work on this objective to date has focused on building the molecular bridge that will allow us to detect the binding of an agent to antibodies that are anchored to a glass slide. The same technology will be used to attach antibodies to the carbon-coated slides. As seen in Figure 9, multiple layers are required for the attachment of the antibodies to the glass slide. The glass is first cleaned with water and then ethanol before applying a thin poly-D-lysine coating, which provides a positively charged surface. This step is necessary to allow other molecules to be bound since few biological molecules can stick to the glass. Next, a layer of protein A, which has a high affinity for the IgG class of antibodies, is added. The advantage of using protein A is that any IgG antibody will readily attach to the protein A and thus, a large number of antibodies can be bound in this manner. Many of the antibodies will bind to the protein A in an orientation that favors binding of a particular ligand or molecule. In traditional microarrays, the ligand or binding molecule needs to be fluorescently-labeled in order to detect its binding to the antibodies.

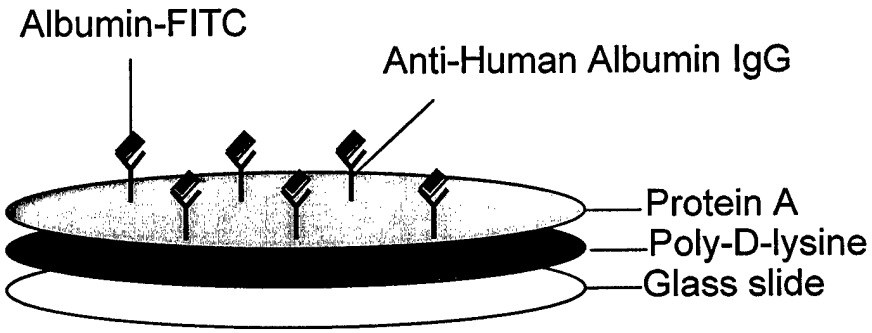


**Figure 9:** Side View - Antibody Array

In pilot studies using glass slides, we utilized fluorescently-labeled antibodies and binding molecules to test the specificity of our method of constructing an antibody array. Binding of the fluorescent molecules was detected using an Olympus fluorescent microscope and photographed using a Nikon digital camera. We found that the addition of poly-D-lysine and then protein A allowed the detection of fluorescent-labeled IgG antibodies (Figure 10A). These data provide evidence that antibodies could attach to the protein A as predicted. When the antibodies were not included in the array, then no signal was detected even in the presence of a fluorescently labeled binding molecule (Figure 10B). Similarly, if the antibodies were present but the binding molecule was not included then no signal was detected (Figure 10C). However, if antibodies and binding molecules were included (Figure 10D), a strong signal was detected. These results demonstrate that the design of our antibody microarray allows for the detection of a specific binding molecule to the antibodies. We found that the intensity of the signal, which corresponds to the amount of fluorescent molecule bound by the antibody, is proportional to the amount of binding molecule presented to the antibodies (Figure 11 A and B). Taken together,

our data provide evidence that validates the methodology used for building the antibody microarrays to detect specific molecular binding events. While the current method will allow the detection of a small number of samples, it will be necessary to build microarrays that contain hundreds or even thousands of antibodies.

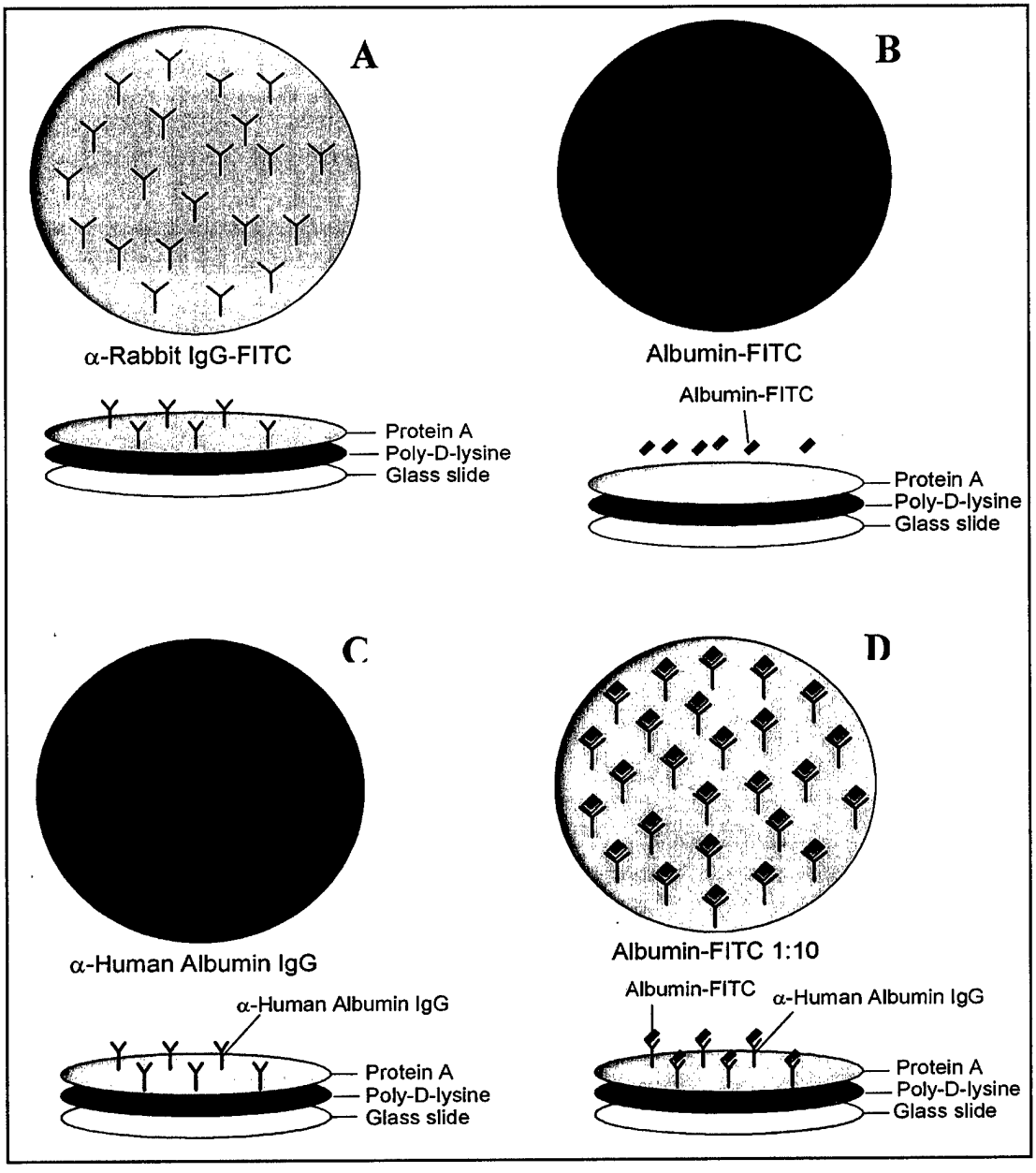


**Figure 10**

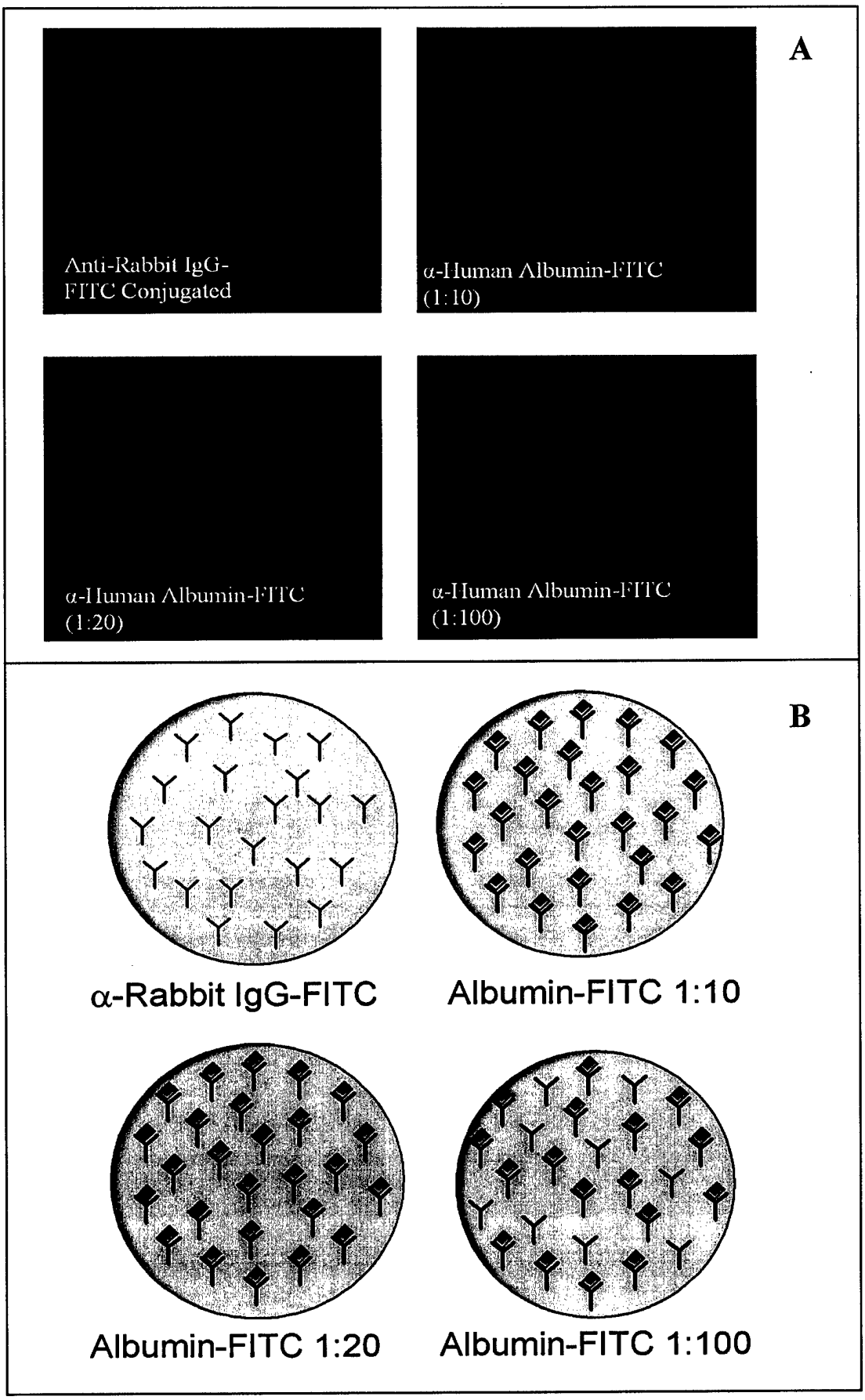
A
Anti-Rabbit IgG-
FITC Conjugated
α-Human Albumin-FITC
(1:10)
α-Human Albumin-FITC
(1:20)
α-Human Albumin-FITC
(1:100)
B
α-Rabbit IgG-FITC
Albumin-FITC 1:10
Albumin-FITC 1:20
Albumin-FITC 1:100

**Figure 11**

## e. Air collection system

The primary objective of Task IV – Final Design and Testing is to develop build a working prototype of a CPMEMS generic bio-detection instrument. This instrument will incorporate a MEMS type collection mechanism [4, 5] to survey a macroscopic volume of air or aqueous solution. Preliminary work on a prototype antigen collection system has been done and will be discussed below.

A crucial step in the detection of biological agents is bio-aerosol collection, collecting particles from the air. All bio-aerosol collection systems follow a similar chronology during the course of their operation: (1) air intake, (2) interface between air and capturing material, and (3) sample transport to detector. In order to design the most efficient collection system, the parameters of these three stages must be optimized. The air intake should be large enough to ensure that if biological material is present in the air, then the material will enter the detector. The interface between the air and the capturing material must also be large so that every antigen present in the air has an opportunity to be captured. The collected material must be transported quickly to the detector and in a concentrated medium for quick and easy detection.

When considering current technology, there is a need for a real-time system in which detection occurs immediately after the antigens are captured. Developing real-time collection and the optimization of the three stages is the aim of our prototype system. The model for our research comes from the human body. In general the respiratory system reveals that it optimizes all of the collection stages. We breathe 2 gallons of air every minute. The combined surface area of the inner walls of the brachial tubes is about 70 square meters. The moist atmosphere of the lung tissue ensures that biologics are captured and enter the blood stream quickly.

Using the respiratory system as a model, we are currently designing/building a system that should achieve real-time collection. It operates as follows and is shown schematically in Figure 12. Water vapor is produced in a chamber and is sent into an interface chamber. The thousands small water droplets provide ample interface area on which the biological constituents of the air can become attached. A continuous flow of air is pumped in and brought into contact with the water vapor. The air/water vapor mixture is then sent directly to a condensing chamber utilizing a thermoelectric cooler. Once in liquid form the concentrated sample will be exposed to the detector.

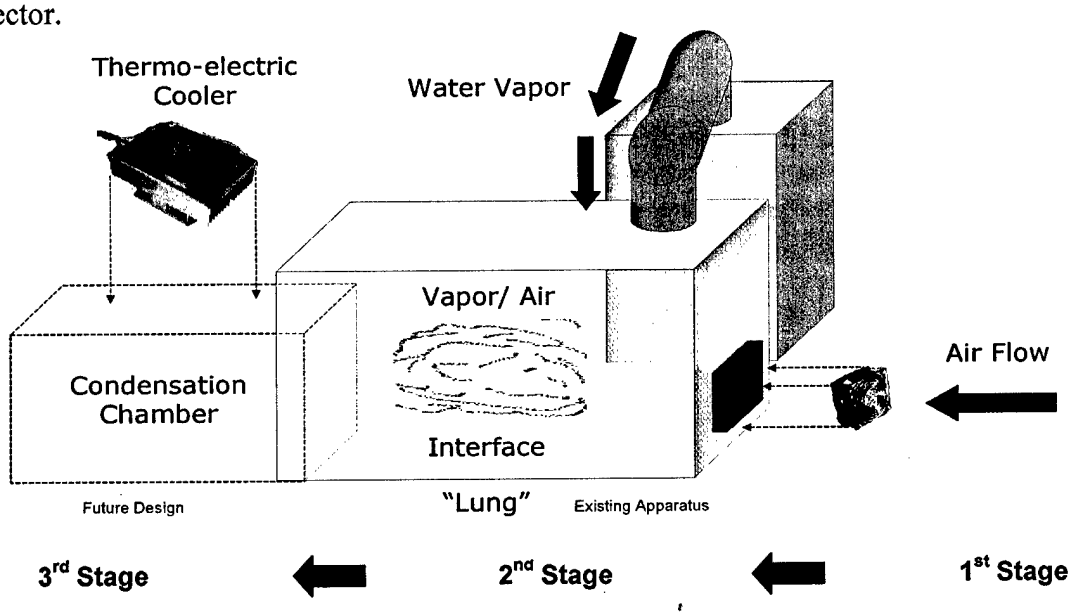


**Figure 12:** Schematic representation of an antigen collection system

## IV. Conclusions

### a. Accomplishments and future work

During the time frame that this interim report covers we have:

1. Completed a study on a carbon/polymer/metal cluster compositions using poly(styrene-co-acrylonitrile) and gold. The microstructure and electrical measurements indicate that these materials can be tailored to a transition region where there electronic properties are critically dependent on vibrations or surface acoustic waves (SAW).

2. Demonstrated the ability, utilizing the JEOL 6360LV SEM, to produce micron sized structures with microlithography processes.

3. Demonstrated the ability to image the surface of Au/PSA composite materials, utilizing AFM, which will be used in the construction of the CPMEMS.

4. Produced data that provide evidence that validates the methodology used for building the antibody microarrays needed to detect specific molecular binding events.

5. Produced an initial prototype design of an antigen collection system.

In the months ahead, our work will include:

1. Within Task I – Device Fabrication, future work will focus primarily on optimizing the microlithography processes to manufacture the CPMEMS and the optimization of device dimensions and composition for bio-sensitivity and bonding with biomaterials.

2. Within Task II – Antigen Platform Feasibility, future studies will involve the use of commercially available kits for designing and constructing antibody microarrays. This will greatly increase the efficiency and reduce the variability in the preparation of the microarrays. We will be using the MicroCaster system from Schleicher & Schuell Bioscience to array the antibodies onto the FAST slides. The experimental conditions will be optimized initially using the glass slides and fluorescent based method. Later, the arrays will be constructed on the carbon-coated slides required for the micro-electromechanical systems. The sensitivity and specificity of the molecular interactions will be determined and compared to the fluorescent based systems.

3. Within Task III – Developing Partnerships, efforts will be made, through the Office of Naval Research, to develop partnerships with Department of Defense Laboratories that will enable the testing of the CPMEMS with priority biological agents.

4. Within Task IV – Final Design and Testing, future work will involve the completion of an antigen collection system that is capable of surveying a macroscopic size sample. Design of instrumentation to stimulate vibrations and measure electronic signals in the CPMEMS structures will also be priority.

## IV. Notes


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